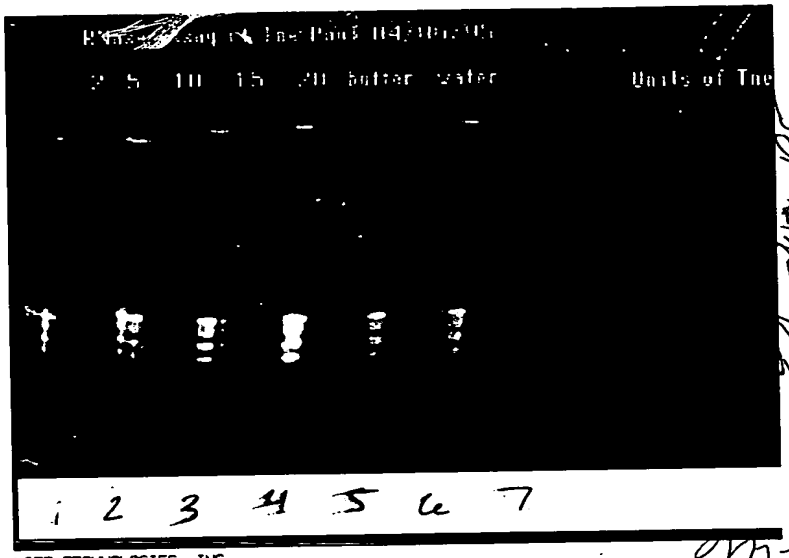
Project No._____
122 Book No._____ TITLE Completion of RNase Assay -

From Page No.__

Take samples from -20°c freezer - spin in micro centrifuge 15 minutes - decant etoh - air dry pellets - Add - ul of RNA blue juice - heat 30 m sec at 90°C Run out on 6% sequencing gel - 400 volts -



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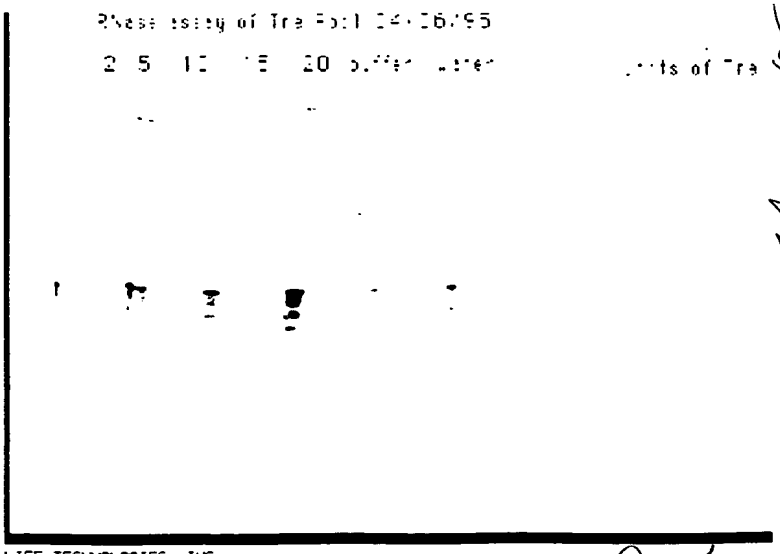
ET 04/04/95

Conclusion - Appears to be RNase free! Next time use more RNA - Only used half of recommended amount Used 1ug vs recommended 2ug.

Bradford on Pools

ET 04/04/95



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Witnessed & Understood by me,	Date	Invented by E. Flynn	Date
Mary Longo	4/13/95	Recorded by	04/04/95

Exonuclease Assay - Tne Pool

Page N .__

E. No. 30042 SOP -

Tube	Rxn Mix 45	Enzyme Units	µl	H_2O
1		~~0~~ 0	—	5 µl
2		~~10~~ 2.0	←	4 µl .5U/µl
3		~~20~~ 5	1	
4		10	2	
5		15	3	
6		~~20~~	4	
7		0		5 µl dil'n buffer

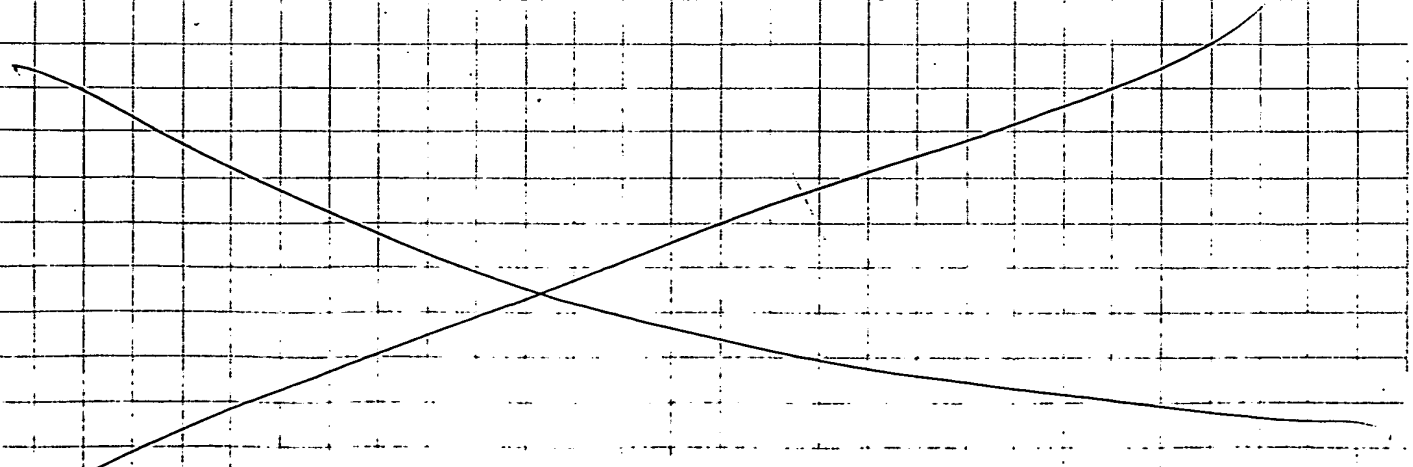
Rxn Mix

10x PCR	80
50mM $MgCl_2$	80
5' ds sub	16 pmol 32 µl .5 pmol/µl
3' ds sub	16 pmol 32 µl .5 pmol/µl
H_2O	494
	720

16 rxns -

heat @ 37°C for 1 hour - 1-7
heat @ 72°C for 1 hour - 8-14

see page - 126 for data



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Mary Longo	4/13/95	Recorded by	04/[illegible]/95

[redacted]

Project No.______
Book No.______
TITLE Endo Assay - 18038 QCP_Ta

From Page No.__

Rxn Mixture - for 8 rxns - (all tubes on ice before use -)

	10x PCR buffer -	40 µl
	50mM MgCl2 -	40 µl
	→ ϕX174 ⊕ DNA -	8 µg (23.5 µl) ✓
15264-0B	Autoclaved H2O	256.5
.34 µg/µl		
		360 µl

	Endo Mix	H2O	Diluted enzyme 5U/µl
1	45	5	—
2	45	1	2 units - 2/µl
3	45	4	5 units - 1µl
4	45	3	10 units 2µl
5	45	2	15 units 3µl
6	45	1	20 units 4µl
7	45	5 Dil'n Buffer ✓	

Incubate @ ~~72°C~~ EKF 37°C for ~~3~~ hours - 5.5 hours

Taq

Double Strand Assay -

	10x PCR buffer	40 ✓
	50mM MgCl2	40 ✓
25260-027	- ϕX174 RF	8 ~~[illegible]~~ 24.2 ✓
EF ~~[illegible]~~ 1702	Autoclaved H2O	2 ~~5~~5.8
.33 µg/µl		
		360 -

	Endo	H2O	Dil Enzyme	5U/µl
1	45	5		
2	45	1	2	4µl of .5U/µl
3	45	4	5	1µl
4	45	3	10	2µl
5	45	2	15	3µl
6	45	1	20	4µl
7	45	5 Dil'n buffer ✓	—	

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Mary Longo	4/13/95	Eliz. [illegible]	04-04-95
		Recorded by	

Pag No.

Spin samples down add 5 μl of Blue Juice -
Run out on 1.2 % Agarose gel -

1 2 3 4 5 6 7 8 9 10 11 12 13 14

SSingle Endo DS - Endo

H_2O

1	2	3	4	5	6	7	~~8~~	~~9~~	~~10~~
H_2O	2	5	10	15	20	B			

8	9	10	11	12	13	14
H_2O	2	5	10	15	20	B

conv

C = 40 / 60 at 10 u - 4.5 / 1.0 / 4.5 = 5 H / 10 % [illegible]

OW 4/13/95

- Endo looks good - however DS Endo - shows conversion to linear but this is also present in the buffer only lane - it could just be a contaminant in the Dil'n buffer -

Dil'n Buffer used - from A.G. (taken from the 4°C Deer cooler - orange tip -

Conclusion: - free of SS Endo nuclease - possible some DS endo nuclease but control with buffer only shows significant conversion to ~~form~~ linear so believe ~~this~~ the dil'n buffer is or has DS endo ~~also~~ activity not the enzyme prep

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Witnessed & Understood by me,	Date	Invented by	Date
Mary Longo	4/13/95	S. Flynn	04-06-95
		Recorded by	

[redacted]